the reference range (<4 μg per liter), a change from 1.8 to 2.9 μg per liter in a year's time could signal the presence of prostate cancer. If the PSA test selected is reliable over that period of time, this new approach allows for the early detection of cancers at a more curable stage than would have been possible with conventional criteria. It is now recommended that PSA velocity should be determined from three consecutive measurements within a year. A PSA velocity test showing a consistent increase of 0.75 μg per liter per year or greater, based on three consecutive determinations, is suggestive of cancer and warrants further evaluation. Both new approaches point out that PSA concentrations can be in the normal range even in the presence of cancer. Consequently, an accurate and precise determination of PSA levels within the normal concentration range will be required to take advantage of these two new methods.

One area that has not received enough attention is the use of age-adjusted reference values, especially the normal reference range for people at an older age. An age-specific reference range for serum PSA would possibly detect organ-confined prostate cancers earlier in younger men (men with a life expectancy longer than 10 years) at a time when the tumors are possibly more susceptible to cure. Based on the new reference ranges, fewer cancers in older men—who might have clinically insignificant tumors or have a less than ten years' life expectancy and not benefit from surgical treatment—will be detected. In the past, 4 μg per liter was the upper normal cut off for men at all ages. The recommended age-specific serum PSA reference ranges are 0.0 to 2.5 μg per liter for men 40 to 49 years, 0.0 to 3.5 μg per liter for men 50 to 59 years, 0.0 to 4.5 μg per liter for men 60 to 69 years, and 0.0 to 6.5 μg per liter for men 70 to 79 years. Studies have indicated that the new age-specific serum PSA reference ranges appear to make PSA a more sensitive tumor marker for men younger than 60 years and a more specific tumor marker for men older than 60 years.

Prostate-Specific Antigen Composition in Serum

Prostate-specific antigen has recently been found to be a serine protease capable of complexing with various protease inhibitors. Consequently, PSA is not free in the blood circulation, but exists largely as PSA complexes with protease inhibitors. The major form of PSA complexes found in the serum is PSA–α_1-antichymotrypsin. The ratio between PSA–α_1-antichymotrypsin and free PSA in the serum is not constant; the ratio increases with increasing concentrations of total PSA. Most important is the finding that the percentage of PSA–α_1-antichymotrypsin complex of the total serum PSA is higher in patients with prostatic cancer than in those with benign prostatic hyperplasia. Therefore, the PSA–α_1-antichymotrypsin value has a higher sensitivity for cancer than the assay for total PSA. In other words, the free serum levels of PSA are substantially lower in patients with untreated prostate cancer than in those with benign hyperplasia; the free serum PSA concentration does not correlate with the total serum PSA or PSA–α_1-antichymotrypsin.

Unfortunately, all current PSA kits were designed to measure free PSA in the serum. Also, PSA values produced by different commercial kits are not always compatible. There are two major reasons for these discrepancies: antibodies used in various commercial PSA kits do not have the same specificities for both free PSA and PSA–α_1-antichymotrypsin complex in the serum, and the calibrators used in various kits not only are not identical to each other, but also do not match the PSA composition found in patients' serum. It was suggested from many studies that the lack of a uniform calibrator among various commercial kits largely accounts for the different PSA values produced by different kits on the same specimens.

New-Generation Assay

A logical improvement for the current PSA kits is to change the focus from the measurement of total PSA to that of PSA–α_1-antichymotrypsin complex, because there is an excellent correlation between serum concentrations of PSA–α_1-antichymotrypsin and total PSA in random and in serial specimens. Therefore, using an assay that specifically measures the PSA–α_1-antichymotrypsin complex in the serum not only simplifies the preparation of a calibrator, but eliminates the difficulty of antibody selection. The new-generation assay, which will be available soon, will also improve the test's specificity for prostate cancer.

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Specimen Quality and Accuracy of Fine-Needle Aspiration Biopsies

OVER THE PAST 20 years, fine-needle aspiration biopsy (FNAB) has become an increasingly popular tool in the United States for diagnosing palpable and—with the adjunctive use of computed tomography (CT) or ultrasonography—deep-seated lesions arising in many body sites.

The accuracy of fine-needle aspiration as an effective diagnostic tool has varied greatly in recent reports: the sensitivity of FNAB to detect malignant neoplasms has ranged from 65% to 98% and the specificity (the ability to rule out malignant neoplasms unequivocally) from 34% to 100%. Looking at these results, the questions are why there is such a vast discrepancy and whether the test has use. In well-trained, experienced hands, however, FNAB is an accurate, cost-effective, and well-tolerated diagnostic procedure.

The principle of FNAB is simple, but its appearance is deceiving, leading to many practitioners doing FNABs without proper training or enough practice to maintain effective skills. Three components are necessary to obtain an accurate diagnosis: adequate specimen sampling, proper specimen preparation, and accurate microscopic interpretation. It is generally well recognized that special skill is required to interpret these specimens, but the importance of specimen quality is less well known, especially among clinicians. In analyzing the literature, it is clear that whereas mistakes at the microscope are the culprits in some inaccurate diagnoses, suboptimal lesional sampling and preparation are responsible for the majority.

In recent years, the increased interest in FNAB among pathologists has led to a growing number of residency training programs that include training in cytologic examination of fine-needle aspirations. A growing number of postgraduate education programs are being offered, and several institutions are offering fellowships in cytopathology. All of this has increased the level of experience in the community.

A good way to institute proper FNAB "basic training" is to teach the basic sampling and smearing techniques on bench material, such as animal liver or excised tumor tissue. Two supervised sessions, with time for practice in between, should prepare trainees for doing supervised FNABs in clinical situations. At least 100 to 200 cases within a six-month period are necessary to achieve an acceptable level of proficiency with the procedure, enabling a practitioner to produce diagnostic material in more than 95% of cases. All cases should be reviewed microscopically by the operator, and at least 25% of the procedures should be supervised, especially difficult cases. To maintain this level of skill, the operator should then do at least 20 biopsies per month.

In this manner, adequate tissue sampling techniques can be used, rendering FNAB a highly accurate, cost-effective method of diagnosis. When integrated into the "triple diagnosis for breast disease," for example, FNAB findings are considered along with mammographic findings and the clinical impression. If the FNAB reveals significant atypia and the clinical impression or mammogram indicates possible cancer, the patient is referred for open biopsy; all other patients are observed clinically. This method reduces morbidity, enhances turnaround time, and the per-case savings is about $700 or, for all breast cancer patients in the United States, a savings of close to $400 million, with a false-negative rate of about 1%.

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Molecular Pathology and Cancer Genetic Screening

WITH THE RECENT, highly publicized discovery of the gene *BRCA1* and its relationship to the familial susceptibility to breast and ovarian cancer, the field of diagnostic molecular pathology has moved into the public consciousness. Along with other important discoveries, such as the genes related to hereditary polyposis and nonpolyposis colon cancer, gene mutations in multiple endocrine neoplasia type II (MEN II), and the linkage of a tumor-suppressor gene (*p53*) to many cancers, molecular oncology is emerging as a vital area in the diagnosis and clinical treatment of neoplasia. These discoveries have brought excitement, but also, with closer scrutiny, trepidation.

Cancer, with its specific chromosomal and nucleotide aberrations, is now being viewed as a true genetic disease. The simplistic hope that a single mutation causes a defined disease has, however, been replaced by the realization that inherited diseases carry mutations unique to themselves. In diseases such as familial breast cancer, familial colon cancer, and MEN II, there are a myriad of mutations. In only a few circumstances does a predominant mutation evolve that lends itself readily to standard molecular diagnostic testing.

Multiple endocrine neoplasia type IIA (MEN IIA), an autosomal dominant and nearly fully penetrant hereditary cancer syndrome, leads to the development of medullary thyroid cancer, pheochromocytoma, and parathyroid hyperplasia. The gene causing MEN IIA, the *RET* proto-oncogene on chromosome 10, functions as a receptor tyrosine kinase. Mutations in the *RET* proto-oncogene are all point mutations; 93% occur in two hot-spot regions in the gene. By using the polymerase chain reaction (PCR), an efficient and inexpensive tool, defined regions of the *RET* proto-oncogene containing the hot-spot regions can be amplified. Mutations change the DNA sequence and create or abolish restriction endonuclease cleavage sites. If restriction endonucleases are judiciously chosen, the PCR product can be digested and the fragments can be size-fractionated. These digest patterns can distinguish affected persons. In fact, each specific mutation can be identified with this technology. Laboratories often begin screening for possible MEN IIA mutations by examining the two hot spots; through these screens, 93% of all disease carriers can be identified. If this search yields a normal pattern, a more stringent analysis is begun by using the same technology at less frequently mutated regions of the gene. As many as 95% of all disease carriers can be detected by this approach. Once a mutation is identified in a family, other members of the kindred can then be screened with 100% sensitivity. With early diagnosis, affected persons can be treated